

THE CYTOGENETICS OF FACULTATIVE APOMIXIS IN MALUS SPECIES

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SOME OF THE ASIATIC SPECIES OF *Malus* produce predominantly maternal progeny, even when surrounded by other species which bloom at the same time. Since apple species and varieties are generally self-sterile, the production of uniform progeny from trees surrounded by other species or varieties would indicate that these trees are apomictic. The production of occasional variants and the production of occasional hybrids following artificial cross-pollination indicate that apomixis is facultative in these species.

Among the facultative apomicts are *Malus sikkimensis* from northern India, the related *M. rockii* from western China, *M. toringoides* and *M. hupehensis* from China and *M. sargentii* from Japan.

Several of these apomictic apple species are excellent ornamental trees, and *M. toringoides* and *M. sargentii* are usually propagated from seed. For many years the apomictic *Malus* species have been tested as rootstocks for ornamental and horticultural apple varieties. If such seedlings proved to be compatible with the clonal varieties they would provide uniform rootstocks without the expense and long period of time needed to obtain uniform rootstocks by vegetative propagation.

During the past twenty years we have used seedlings of *Malus sikkimensis* as rootstocks for apple varieties. They have proved to be compatible with all ornamental and commercial varieties of apples tested, and for some varieties they are semi-dwarfing. The first seedlings used were from a tree (AA No. 17459) which was raised from seed obtained from the Royal Botanic Gardens, Kew, England, in 1902. The progeny were relatively uniform with only an occasional variant. Unfortunately this tree was cut down before chromosome counts were obtained. The only other source of seed until recent years was a seedling of AA 17459 which was planted in 1936 (AA 50-36-A). Seedlings from the daughter tree proved to be much more variable than those of the original tree.

Malus rockii in the Arnold Arboretum is represented by a tree (AA 8334) grown from seed obtained from the University of California Botanic Garden in 1936. Like *M. sikkimensis* it is slow to come into fruit, and seed has been available only during the past few years. The two-year-old trees in the nursery are very uniform, varying only in size and vigor.

Malus hupehensis (AA 7441) has given the most uniform progeny of all the species tested, but the seedlings are not very compatible when budded with horticultural apple varieties. The occasional variants are usually dwarf forms.

The oldest tree of *M. toringoides* in the Arboretum (AA 17475) is from seed collected in China by Wilson in 1908. It has produced relatively uni-

form progeny and mature seedlings grown on the Peter Bent Brigham Hospital grounds are duplicates of the mother tree. But seeds sent to Stern in England produced a variant with larger fruit, which was given the varietal name *macrocarpa*. This variety, which is represented in the Arnold Arboretum (AA 11246), flowers about a week earlier than does the mother tree. Seedlings of var. *macrocarpa* grown at the Case Estates of the Arnold Arboretum by Dr. Wyman proved to be very variable in size and, in the several which have borne fruits, these differ in size and shape from either the species or the variety.

The oldest specimen of *M. sargentii* (AA 4681) in the Arnold Arboretum was grown from seed collected in Japan by Sargent in 1892. The variety *rosea* originated as a seedling of the species grown in the Rochester Park. A specimen (AA 11045) was planted in the Arnold Arboretum in 1921. Both *M. sargentii* and var. *rosea* produce 90 per cent or more of maternal-type seedlings when open pollinated. The variants usually differ from their parents primarily in size and vigor. Seedlings of the variety are more vigorous and are more compatible when budded with horticultural varieties than are the seedlings of the species. The variety is being tested as a dwarfing rootstock.

Chromosome counts of these apomictic species and their progeny have been obtained from meiotic divisions of the pollen mother cells and from somatic tissue. The mitotic divisions were obtained from parenchyma cells developed from the medullary rays. A strip of bark was removed from the trunk or branch of the tree and the exposed wood was covered with a strip of polyethylene film. The parenchymatous tissue from the medullary rays develops rapidly and after five or six days can be cut off in thin ribbons and fixed in alcohol-acetic fixative and stained in aceto-carmin. Although the large parenchyma cells provide much better chromosome figures than do root-tip or leaf-tip smears, exact counts are difficult or impossible in the triploids and tetraploids. In most cases the somatic counts reported are accurate to ± 1 or 2. Meiotic counts, particularly in the triploids, are also subject to some error, because of variation in the degree of pairing of the meiotic chromosomes and the great irregularity of meiosis in most of the triploids.

A summary of the chromosome counts is shown in TABLE I. The seedling of the original specimen of *M. sikkimensis* grown in the Arnold Arboretum is a triploid, but it produces both triploid and tetraploid progeny. A meiotic anaphase of one of the tetraploid progeny is shown in FIG. 1. Two seedlings of one of the tetraploid segregates grown in the Bussey Orchard (BO 4), were examined for chromosome counts and both were found to be triploid. The tetraploids generally have larger flowers and fruits than do the triploids and bloom earlier in the spring.

Malus rockii is a tetraploid and relatively good meiotic figures were obtained (FIG. 2). At the first meiotic division there are often several univalents, but the division is quite normal. The seedlings of *M. rockii* grown in the nursery included both tetraploids and, apparently, pentaploids, although only 76 chromosomes could be found in the somatic figure illus-

trated (FIG. 12). Some of the somatic counts reached 85, but exact counts were impossible.

TABLE I. Chromosome Counts of Facultatively Apomictic *Malus* Species and their Progeny

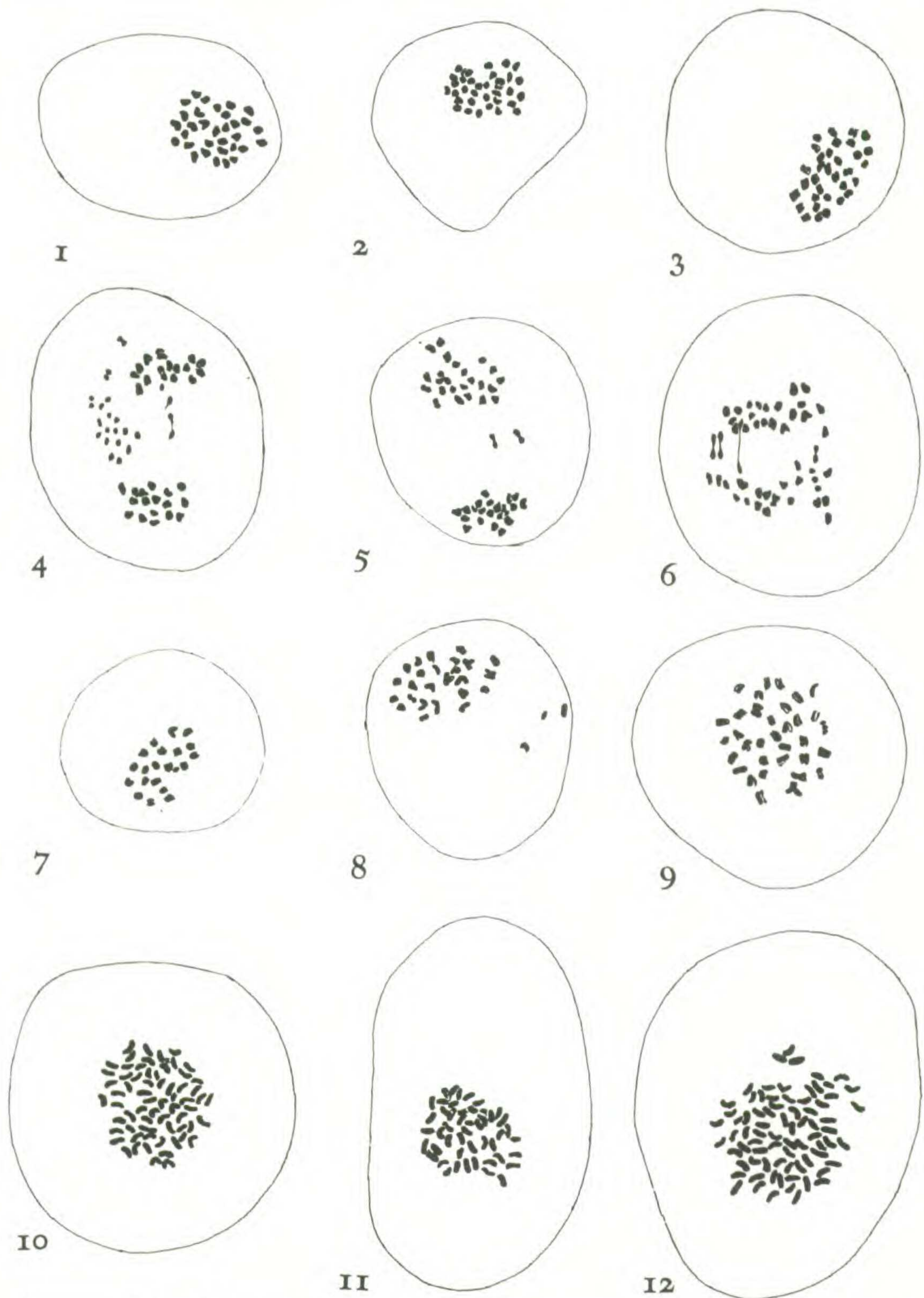
SPECIES	RECORD NUMBER OF SEED PARENT		CHROMOSOME NUMBERS PROGENY		
	SEED PARENT	SEED PARENT	$3n$	$4n$	$5n$
<i>M. sikkimensis</i>	AA 17459	?	1	7	
<i>M. sikkimensis</i> sdlg.	BO 4	$4n$	2		
<i>M. rockii</i>	AA 8334	$4n$		4	2
<i>M. hupehensis</i>	AA 7441	$3n$			
<i>M. toringoides</i>	AA 17475	$3n$			
var. <i>macrocarpa</i>	AA 11246	$4n$	6		
<i>M. sargentii</i>	AA 4681	$4n$		3	
var. <i>rosea</i>	AA 11045	$3n$	3		

Malus hupehensis is a triploid, as determined from meiotic counts by Dermen (1936) and from our somatic counts. Meiosis in the pollen mother cells is very irregular; the tetrads disintegrate and no mature pollen grains are formed. Among the seedlings of *M. hupehensis*, Dermen found two tetraploids in 39 seedlings, based on somatic counts in root tips.

Malus toringoides also has very irregular meiotic divisions of the pollen mother cells and stages from prophase to tetrads can be found in a single anther. No critical counts could be obtained from meiotic figures, but somatic counts show that this species is a triploid. The var. *macrocarpa*, however, is a tetraploid, as determined from both meiotic and somatic counts. Meiosis in the tetraploid is normal with only occasional lagging univalents. All the seedlings of the variety which were examined were triploids.

Malus sargentii is a tetraploid with about 34 chromosomes at meiosis (FIG. 3) and 68 chromosomes at mitosis. The seedlings from the Arnold Arboretum trees are also tetraploids, but the fact that this species gave rise to the triploid var. *rosea* indicates that some triploid segregates would be found if enough seedlings were grown. The var. *rosea* is a triploid with many univalents at the first meiotic division (FIG. 4). Only three seedlings of the variety were examined cytologically and all were triploids. It is probable that some of the larger progeny of var. *rosea* are tetraploids, but these were not examined.

Many hybrids have been made between *M. sargentii*, or var. *rosea*, and diploid ornamental apples to combine the dwarf growth habit of this species with the superior flowering and fruiting characters of the ornamental diploid varieties. Crosses have also been made with 'McIntosh' as the first step in combining the disease resistance of *M. sargentii* with the fruit size and quality of 'McIntosh.' *Malus sargentii*, or its var. *rosea*, when artificially



FIGURES 1-12. Meiotic and mitotic chromosomes of *Malus* species and hybrids. 1, *M. sikkimensis*, meiotic anaphase I, 32 chromosomes. 2, *M. rockii*, meiotic anaphase I, 35 chromosomes. 3, *M. sargentii*, meiotic metaphase II, 33 chromosomes. 4, 5, *M. sargentii rosea*: 4, meiotic anaphase I, $3n$; 5, meiotic anaphase I, $3n$. 6, *M. sargentii rosea* \times 'McIntosh' (5750), meiotic anaphase I, $3n$. 7, *M. sargentii rosea* \times *arnoldiana* (4650), meiotic anaphase I, 21 chromosomes. 8, *M. sargentii rosea* \times *astracana* (33340), meiotic anaphase I, $3n$. 9, *M. sargentii rosea* \times *astracana* (33340 seg.), meiotic metaphase II, $4n$. 10, *M. sargentii*

pollinated with pollen of a diploid produces from 0 to about 25 per cent of sexual hybrids. Most of the progeny are maternals in all crosses.

The hybrids were grown to maturity and their progeny from open-pollinated seeds were tested for breeding behavior. Most of them tend to breed true, with a small proportion of variants, indicating that facultative apomixis is a dominant character. The cytology and breeding behavior of the *M. sargentii* hybrids is summarized in TABLE II.

The pollen parents were diploid species and varieties which, when open pollinated, produced extremely variable progeny. Hybrid 19039 ('Henrietta Crosby') is a cross between *M. arnoldiana* (*M. floribunda* × *M. baccata*) and *M. pumila* var. *niedzwetzkyana*; 20139 is *M. arnoldiana* × *M. purpurea* f. *eleyi* (*M. pumila* var. *niedzwetzkyana* × *M. atrosanguinea*); and 6639 is an open-pollinated segregate of *M. spectabilis* f. *riversii*. *Malus astracanica* is a hybrid between *M. pumila* and *M. prunifolia*, and the Hopa Crab is *M. baccata* × *M. pumila* var. *niedzwetzkyana*. As would be expected, the progeny of these hybrids are very variable.

The sexual hybrids produced by crossing *M. sargentii* with 19039 or 20139-2 are triploids, as might be expected (FIG. 5). However, when pollinated with 20139-1 or 20139-3, *M. sargentii* produced aneuploid hybrids with the chromosome number near the diploid level. Hybrid 5650-2 had about 21 bivalents with only occasional univalents (FIG. 7). These hybrids lacked vigor and did not live long.

Crosses between the triploid *M. sargentii* var. *rosea* and the diploid species produced progeny ranging from near-diploid to tetraploid. The hybrid between var. *rosea* and *M. astracanica* was a triploid (FIG. 8). Progeny of this hybrid (33340) produced both triploids and tetraploids (FIG. 9). The tetraploid had larger flowers and fruits and bloomed earlier than did the original hybrid.

When crossed with *Malus arnoldiana*, *M. sargentii* var. *rosea* produced an aneuploid with about 17 bivalents and several univalents at meiosis. An aneuploid also resulted when var. *rosea* was crossed with 6639, but two sister hybrids were approximately triploid. Crosses with 'McIntosh' produced a triploid (FIGS. 6, 11), and three tetraploids or near tetraploids (FIG. 10). Crosses with Hopa Crab produced a tetraploid and two feeble aneuploids.

Progeny tests of the *Malus sargentii* F₁ hybrids indicate that facultative apomixis is a dominant character. The F₁ trees were grown in test plots with diploids and the open-pollinated seeds of the hybrids were presumably pollinated with pollen of both diploids and polyploids, although pollen fertility in the triploids is low. The seeds were stratified and grown in flats in the greenhouse. Some of the progenies were transferred to the nursery for further observation and selection. The percentage of variants was somewhat higher when the progenies were scored as young seedlings,

var. *rosea* × 'McIntosh' (5750-B), mitotic metaphase, 67 chromosomes. 11, *M. sargentii* var. *rosea* × 'McIntosh' (5750), mitotic metaphase, 48 chromosomes. 12, *M. rockii* segregate, mitotic metaphase, 76 chromosomes.

because some of the variants were weak plants which did not survive or were too small to transfer to the field. In the field there was some variation in size and vigor of the maternal types. The variant seedlings differed from the maternal type in leaf characters and growth habit and generally were quite variable. The proportion of maternals and variants produced by some of the hybrids is shown in TABLE II.

All of the triploids and most of the tetraploid hybrids produced predominantly maternal progeny when open pollinated. However, one of the tetraploid hybrids between *M. sargentii* var. *rosea* and 'McIntosh' produced twice as many variants as maternals. The tetraploid segregate of *M. sargentii* var. *rosea* × *M. astracana*, 33340, also produced a high proportion of variants, as did the tetraploid segregates of the triploid species *M. sikkimensis* and *M. toringoides*. Yet the tetraploid *M. sargentii*, when open pollinated, produces nearly 100 per cent of maternals.

TABLE II. Cytogenetics of Malus Species Hybrids

CROSS	RECORD NUMBER	CHROMOSOME COUNTS		PROGENY SEGREGATION	
		MEIOTIC	MITOTIC	MATERNAL	VARIANTS
<i>Malus sargentii</i>	AA 4681	$4n$	$4n$	+	
× 19039	4751-1	$3n$	$3n$	40	2
× 20139-2	4851-1	$3n$		12	0
× 20139-2	4851-2	$3n$			
× 20139-1	5650-1	$16-17_{II} + 1-3_I$			
× 20139-1	5650-2	$20-21_{II} + 1-3_I$			
× 20139-3	5650-3	$16-17_{II} + 1-3_I$			
<i>M. sargentii</i> var. <i>rosea</i>	AA 11045	$3n$	$3n$	259	24
× <i>astracana</i>	33340	$3n$	$3n$	25	2
Segregate of 33340		$4n$	$4n$	28	14
× <i>atrosanguinea</i>	17039	$3n$		72	3
× <i>arnoldiana</i>	4650-3	$17_{II} + 3_I$			
× 6639	5150-0	22_{II}			
× 6639	5150-1	$3n$			
× 6639	5150-2	$3n$			
× 'McIntosh'	5750-1	$3n$	$3n$	16	1
× 'McIntosh'	5750-2	$4n$	$4n$	8	16
× 'McIntosh'	5750-A	$4n$	$4n$	17	2
× 'McIntosh'	5750-B	$4n$	$4n$	33	1
× Hopa Crab	4450-A	$17_{II} + 2-3_I$			
× Hopa Crab	4450-B	$4n$		72	4
× Hopa Crab	4450-C	$18-20_{II} + 0-4_I$			

DISCUSSION

The facultatively apomictic species of *Malus* include both triploids and tetraploids. The triploids usually produce maternal progeny, presumably as the result of the development of an unfertilized and unreduced egg cell.

The occasional production of tetraploids by triploids would indicate that the unreduced egg cell was fertilized by pollen from a neighboring diploid species. The tetraploid species may breed true with only an occasional variant, as in *Malus sargentii*, but in the tetraploid forms of *M. sikkimensis* and *M. toringoides* the progeny are variable with respect to their morphological characters and, in *M. toringoides*, most or all of the segregates are triploids. Apparently the seeds of *M. sargentii* are derived from an unreduced egg cell, while in the tetraploid forms of *M. sikkimensis* and *M. toringoides* they are often, or perhaps usually, produced from the union of a reduced egg cell and a haploid gamete from pollen of a neighboring diploid species.

The sexual hybrids would tend to resemble the mother plant since in the triploids descended from a tetraploid female parent the latter would contribute two of the three genomes, in the tetraploid descended from a triploid female parent the latter would contribute three of the four genomes, while in the pentaploid *M. rockii* segregates the maternal parent would contribute four of the five genomes. Only in the triploids descended from tetraploid female parents would there be genetic segregation due to the reduction division in megasporogenesis, and then only if the tetraploid is heterozygous.

Often the facultatively apomictic tetraploids may produce some triploids, while the triploids may produce tetraploids, and both types of polyploids will have the major characters of the species. Thus chromosome counts from one or a few individuals may not reflect the true cytological picture. It is probable that this condition may be found in other genera of the Pomoideae. The diversity of types in some of the apomictic species of *Cotoneaster* and *Crataegus*, for example, could be the result of such chromosome irregularities.

In the controlled pollination crosses between the tetraploid *M. sargentii* and diploid species and varieties, the production of triploid hybrids was not unexpected (in view of the breeding behavior of open-pollinated triploid species) but the occurrence of near diploids was not expected. The triploid var. *rosea* also produced some near diploids. Apparently both forms of *M. sargentii* produce some egg cells with near diploid chromosome numbers. It is possible that such near-diploid segregates are produced in open-pollinated segregates, but are eliminated or discarded in the young seedling stage.

Triploid apples crossed with diploids normally produce aneuploid segregates with chromosome numbers ranging from diploid to triploid, regardless of whether the triploid is the female or the male parent, indicating that the triploid parent produced gametes with chromosome numbers ranging from 17 to 34 (Wanscher, 1939). The distribution of chromosome counts of the progeny indicates normal chromosome distribution with the most frequent gametic contributions from the triploid in the 23–25 chromosome classes.

As a result of random distribution of the chromosomes in the meiosis of sexual triploids, the progeny of triploids crossed by diploids usually includes very few diploids or tetraploids, and segregates with 40–42 somatic

chromosomes are the most frequent. Yet the triploid *M. sargentii* var. *rosea*, when crossed with diploids, produced tetraploids, triploids and aneuploids near the diploid level in about equal frequencies. Meiosis in the triploids is irregular and could produce egg cells ranging from haploid to diploid, but meiosis in the tetraploid *M. sargentii* does not indicate much irregularity. There is no evidence from microsporogenesis that the tetraploid could produce microspores with one, two or three genomes such as Gottschalk (1958) has described in tetraploid tomatoes. Yet *Malus sargentii* pollinated with diploid species does produce a few hybrids near the diploid level. Apparently megasporogenesis is more irregular than microsporogenesis in these *Malus* species; however, it has not been studied cytologically.

In the progeny of tetraploid *M. sieboldii* crossed with diploid species, Olden (1953) found an occasional diploid or near diploid segregate, some triploids, and an occasional pentaploid. Although *M. sieboldii* usually produces tetraploid maternals, chromosome reduction can occur to produce near haploid or diploid egg cells and the unreduced egg cell can also be fertilized. Even in cultivated clonal varieties of apples, Einset (1952) finds that diploids may produce triploids and triploids may produce tetraploids by the functioning of unreduced egg cells fertilized by haploid male gametes. More recently he has obtained pentaploids from tetraploids.

Some of the progeny of *Malus rockii* which we have classed as pentaploids have less than 85 chromosomes, as is shown in FIG. 12. Olden found counts of 70, 80 and 82 in progeny from open-pollinated *M. sieboldii*. He also found variable chromosome numbers in some segregates as Hegwood and Hough (1958) have found in the 'Winter Pearmain' apple and its segregates. As a rule, however, the facultatively apomictic tetraploid apple species usually produce tetraploid or diploid egg cells.

The dominance of facultative apomixis in the polyploid *Malus* species is in accord with the inheritance of facultative apomixis in other polyploid genera (Stebbins 1950). This dominance should facilitate the production of facultatively apomictic hybrid segregates which could be propagated from seed.

SUMMARY

Facultatively apomictic species of *Malus* include *M. sikkimensis*, *M. rockii*, *M. hupehensis*, *M. toringoides*, *Malus sargentii* and its var. *rosea*. All are polyploids: the tetraploid species produce maternal tetraploids and hybrid triploids or pentaploids; the triploid species produce maternal triploids and hybrid tetraploids; both tetraploids and triploids also produce some aneuploids near the diploid level. Facultative apomixis is a dominant trait in the F₁ hybrids between the facultatively apomictic polyploids and sexual diploids.

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